Standard Operating Procedure for Zooplankton Sample Collection and Preservation and Secchi Depth Measurement Field Procedures

LG402

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1.0 SCOPE AND APPLICATION

1.1 This Standard Operating Procedure describes the field sampling and preservation of zooplankton samples and the measurement of Secchi depth for the GLNPO open water Great Lakes surveys.

2.0 SUMMARY OF METHOD

2.1 Two sampling tows are performed at each station. One tow is from 20 meters below the water surface to the surface using a 63-µm net. The other tow is from 100 meters below the surface to the surface using a 153-µm net. If the station depth is less than the specified depth, the tow is taken from two meters above the bottom to the surface. The tow net, with a screened sample bucket attached at the bottom, is lowered to the desired depth, and raised at 0.5 meters per second to collect zooplankton from the water column. After lifting the net from the water it is sprayed with a garden hose to wash the organisms down into the bucket. The sample is concentrated into the sample bucket and is transferred to a sample storage bottle. The organisms are narcotized with soda water and preserved with sucrose formalin solution.

3.0 SAFETY AND WASTE HANDLING

3.1 Refer to GLNPO's *Health, Safety and Environmental Compliance Manual* (May 1997, or as amended) and individual instrument procedural operations manuals for specific details on applicable 1) personal health and safety issues; 2) instrumental, chemical, and waste handling procedures; and 3) accident prevention. This applies to all EPA personnel, EPA contractors or federal, state, or local government agencies, and persons who operate or are passengers onboard US EPA GLNPO vessels during all activities and surveys.

3.2 It is the responsibility of the user of this method to comply with relevant chemical disposal and waste regulations as sited in GLNPO's *Health, Safety and Environmental Compliance Manual* (May 1997, or as amended). All applicable safety and waste handling rules are to be followed. All containers storing reagents, standards, controls, blanks, and wastes used in the laboratory must be properly identified through appropriate labeling and hazard definition. Good technique includes minimizing contaminated waste. Over-board discharges of chemical wastes are forbidden.

3.3 Every chemical should be regarded as a potential health hazard and exposure to these compounds should be as low as reasonably achievable. Please refer to Appendix L in GLNPO's *Health, Safety and Environmental Compliance Manual* (May 1997, or as amended) for more detailed descriptions of the potential risks associated with any chemicals used in this method. It is good laboratory practice to wear a lab coat, safety goggles, and gloves at all times.

3.4 During sampling, caution, common sense, and good judgement should dictate appropriate safety gear to be worn in any given situation on deck. Hard hats, gloves, and steel-toed shoes must be worn in working conditions where there is a possibility of injury to the head, hands, or feet; however, if in doubt, please ask the Chemical Hygiene Officer.

3.5 Collecting samples in cold weather, especially around cold water bodies, carries the risk of hypothermia and frostbite. Sampling team members should wear adequate clothing for protection in cold weather. For specific information regarding sampling during cold conditions, please refer to the US EPA GLNPO *Standard Operating Procedures for Winter Operations* (December 1994, or as amended).

3.6 Collecting samples in extremely hot and humid weather carries the risk of dehydration and heat stroke. Sampling team members should carry an adequate supply of water or other liquids for protection against dehydration in hot weather.

3.7 Work vests must be worn while working on the fantail and Rosette deck.

3.8 Formaldehyde is a known carcinogen. During the preservation of samples, the formalin should be dispensed under a hood. A lab coat, gloves, and safety glasses or goggles should be worn.

4.0 EQUIPMENT AND SUPPLIES

Plankton tow net, 63-µm pore size, 0.5-m diameter (D:L=1:3)
Plankton tow net, 153-µm pore size, 0.5-m diameter (D:L=1:3)
Tow net sample bucket with a 61-µm pore size metal screen
Tow net sample bucket with a 151-µm pore size metal screen
Flowmeters - Kahl Scientific Company, 005WA200
Weights, 10-20 lbs.
Safety line for sample bucket
Garden hose
Soda water (club soda)
500-mL plastic sample bottles
Repipettor with 20-mL delivery capability
Graduated cylinder with 50 - 100 mL capacity
Waterproof notebook
Gloves (rubber or vinyl)
Winch with metering sheave and hydrographic line

5.0 REAGENTS

Sucrose (crystalline)
Formalin (37% solution of formaldehyde in water)
Borax (powder)

6.0 BUFFERED SUCROSE FORMALIN PREPARATION

6.1 Dissolve 60 grams of sucrose in 1000 mL of formalin (Haney and Hall, 1973)

6.2 Then dissolve 9 grams of borax in 1000mL of sucrose formalin

6.3 Store in a labeled plastic container

7.0 SAMPLING PROCEDURE

NOTE: *In Lake Superior, because of fewer organisms, two tows must be done for each sample collected with the 63-µm mesh net. The two tows are combined into one sample bottle. Flowmeter readings from both tows are added up and reported as one reading for the combined sample.*

7.1 At each site, the total water depth is obtained from the SeaBird profile.

7.2 The appropriate sample bucket is attached to the net and the net is attached to the winch cable. A rope bridle is clipped to the net frame and extended to the cod end of the net where it is attached to the sample bucket. A weight is added to the lower end of the rope and the bridle is adjusted so that the frame of the net (not the mesh netting) supports the weight.

7.3 The protective cover is removed from the flowmeter which has been mounted slightly off-center in the mouth of the net. The flowmeter number is recorded.An initial reading is taken from the flowmeter. THE FLOWMETER IS NOT ZEROED.

7.4 The winch operator deploys the net so that the rim is at the surface of the water and then sets the cable sheave to zero.

7.5 The cable is deployed until the sheave reads 20 meters (63-µm net) or 100 meters (153-µm net). If the total water depth is less than the specified sample depth, then the rim is only lowered to two meters above the bottom. The net is retrieved at 0.5 meter/second. The sheave reading is checked to be sure that it again reads zero as the net rim clears the water surface. If the cable is slipping or the sheave is not functioning properly, adjustments must be made to the cable metering system.

7.6 The final reading, and average net angle off of vertical during retrieval are recorded.

7.7 The net is rinsed down gently from the outside with ambient temperature lake water to wash all of the organisms off the net cloth and into the sample bucket.

7.8 The collection bucket is swirled gently to concentrate the sample. The bucket is detached and the contents transferred to the sample storage bottle. The bucket is gently rinsed from the outside at least three times. Be sure to leave headspace in the storage bottle to accommodate 40 mL of preservative.

7.9 A final check is made to assure that the sample ID, flowmeter ID, flowmeter reading, cable angle, station code, total depth, depth of tow, date, time, net mesh, and operator code are recorded. The cover is placed over the flow meter and the net is brought inside for storage.

8.0 SECCHI DEPTH MEASUREMENT

8.1 After completion of the zooplankton tows, a Secchi disk transparency measurement should be taken. Secchi disk transparency measurements should always be taken unless the time is between one hour before sunset and one hour after sunrise or if weather prevents collection. If a sampler is unsure of whether or not to collect a Secchi disk measurement, the sampler should consult with the Chief Scientist. When a Secchi disk reading is not collected, the sampler should indicate the reason on the hard-copy field recording form. When entering the Secchi data into GLENDA, the analyst should enter the reason provided on the hard-copy field recording form into the Acomments@ field.

8.2 Unwind an amount of rope from the 30-cm diameter white Secchi disk equivalent to the estimated Secchi depth, plus about five meters.

8.3 Lower the Secchi disk from the shady side of the boat out of direct sunlight, until it is no longer visible.

8.4 Raise the disk slowly until it is just visible again.

8.5 Lower the disk once more until it disappears again. Make sure that you cannot see the disk. Secchi depths in the Great Lakes can be quite deep, and as a result, perspective effects can make the disk appear very small and difficult to see.

8.6 Keeping your eye on the spot on the rope that was just at the surface of the water as the disk disappeared, raise the rope just enough to grab the rope at that spot, and then tow in the disk.

8.7 Measure the length of rope from the disk to the spot you grabbed. This is the Secchi depth. The rope should be marked in meters; estimate the length to the nearest decimeter.

8.8 Field duplicates are taken for Secchi disk measurements each time a field duplicate is scheduled for collection for the surface sample of a lake (the sample collected at 1 meter below the surface). If a field duplicate of a surface sample is not scheduled for a given day, at least one field duplicate Secchi disk reading should be conducted at the station sampled closest to noon. The EPA Shift Supervisor selects the station for the field duplicate reading, which should be performed by the EPA Shift Supervisor and/or Marine Technician. Two different analysts should take the duplicate measurements and the acceptance criteria for these duplicates is less than or equal to 5% of the first measurement + 0.5 meters. Neither technician should know the result obtained by the other technician until the results are recorded. If a duplicate reading fails the acceptance criteria, the two technicians should take another Secchi reading together to determine the cause of the difference.

9.0 SAMPLE PRESERVATION

9.1 The zooplankton samples should be refrigerated as soon as possible after collection. In the shipboard biology lab, 20 mL of soda water is measured with a graduated cylinder, into the sample to narcotize the organisms within 1 hour of sample collection.

9.2 The sample then stands for 30 minutes in the refrigerator.

9.3 Under a hood, 20 mL of sucrose formalin solution is added to the sample.

9.4 The sample storage bottle is filled to the top with reagent water and tightly capped, the cap and neck are wrapped with parafilm to prevent leaks, and the sample storage bottle is stowed in a designated cooler in the walk-in refrigerator.

9.5 All the information recorded during the sampling process from the field notebook is entered into the appropriate data sheet and entered into the computer data base. A printout of the information input is made after each station.

10.0 FIELD QUALITY CONTROL

10.1 Flowmeter Calibration

10.1.1 During each survey season, when calm weather permits, the flowmeter is calibrated. This should be done at the beginning of each cruise if possible. This is accomplished by lowering the rim of the net with flowmeter (without the net cloth) to the 20 meters depth, raising it at 0.5 meter/second, and recording the resulting flowmeter reading. This is repeated 20 times. Readings are recorded on the Zooplankton Net Flowmeter Calibration data sheet and are entered into the computer database. Readings are recorded on the Zooplankton Net Flowmeter Calibration data sheet and are entered into the computer database. The mean flowmeter value of these 20 readings is then used along with the reading during sampling to calculate the net efficiency.

The flowmeter calibrations should be checked again at the middle of the cruise, if possible. Five to ten readings are taken during the calibration check. When recording mid-survey flowmeter calibrations, circle yes in the Mid-survey Flowmeter Calibration section on the field information recording form. If the average of these readings differs by more than 10% from the original calibration readings, and the

differences cannot be explained by sampling conditions (i.e., rough seas or boat is drifting), then the meter needs to be serviced and re-calibrated by taking 20 additional readings.

10.1.2 If the meters begin to give erratic readings that do not correlate with changes in tow depth, line angle, or evidence of net clogging, the chief scientist should be consulted. The meters may need to be cleaned or replaced. Meters should be recalibrated after servicing.

10.2 Cable angle

10.2.1 The cable line of the winch should be nearly vertical to obtain reproducible results. If the angle between the cable line and a vertical line drawn from the top of the cable line to the water surface exceeds 30E during retrieval the sample is discarded, the net is washed and the tow (steps 7.3 to 7.9) is repeated. If weather conditions continually produce drifting of the tow net such that the less than 30E requirement can not be met, the EPA's Shift Supervisor must decide whether to seek haven until proper sampling can be performed.

10.3 Uninterrupted towing

10.3.1 If the tow is interrupted by stopping or changing the winch speed, the sample is discarded, the net is washed, and the tow repeated (steps 7.3. to 7.9).

10.4 The addition of the club soda is performed within an hour of collection.

10.5 The addition of the formalin preservative is performed within two hours of collection.

10.6 If the initial and final metering sheave readings do not correspond to within a meter, corrective action such as using a heavier weight, and/or lubricating the sheave is taken prior to rerunning the tow.

11.0 REFERENCES

11.1 Haney, J.F., and D.J. Hall. 1973. Sugar - coated *Daphnia*: A preservation technique for Cladocera. Limnol. Oceanogr. 18: 331-333.

11.2 Prepas, E. 1978. Sugar frosted *Daphnia*: An improved fixation technique for Cladocera. Limnol. Oceanogr. 23: 557-559.